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**STUDYING THE DISTRIBUTION  
OF ANTHRAX VACCINES IN AN ORGANISM  
BY USING RADIOACTIVE INDICATORS**

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STUDYING THE DISTRIBUTION OF ANTHRAX VACCINES  
IN AN ORGANISM BY USING RADIOACTIVE INDICATORS

Following is the translation of an article by M. M. Agababyan in the Russian-language periodical Izvestiya Akademii Nauk Armyanskoy SSR (Journal of the Academy of Sciences, Armenian SSR), Vol. 12, No 12, 1959, pages 63-71.

Active immunization against anthrax with live, weakened sporous cultures of *B. anthracis* was proposed in 1883 by Professor L. S. Tsenkovskiy, has been applied to the present time, and has yielded brilliant results.

Despite the achievements attained in this field, several problems of the immunogenesis of anthrax have been inadequately developed. Problems such as the period of time that antianthrax vaccines remain in the organism and the fate of these vaccines in the organism are not discussed adequately in the literature. It was determined, however, that the Tsenkovskiy vaccine remains under the skin for 6 to 8 days.

Taking into account the inadequate development of the problem of the fate of the first and second Tsenkovskiy vaccine in the animal organism, we employed the method of marking bacilli with radioactive substances, and studied: (a) the dynamics of distribution and accumulation of marked (radioactive sulfur) Tsenkovskiy vaccine in the normal organism, and (b) the dynamics of distribution of marked anthrax bacilli in the immunized organism.

Resolution of these problems required acquisition of marked bacilli of the first and second Tsenkovskiy vaccine. Radioactive sulfur in the form of methionine was used as the radioactive substance.

To obtain bacilli with the greatest degree of radioactivity we had to find a special nutrient medium which did not contain sulfur, or contained minimal amounts of sulfur, because only insignificant amounts of sulfur had been absorbed by the bacilli when cultivated on ordinary nutrient media containing radioactive sulfur. In our experiments this medium was glycerine-peptone agar [see Note 7]. Glycerine-peptone agar

containing 5.5 microcurie per milliliter of nutrient medium was built up in test tubes, and after bevelling, first and second Tsenkovskiy vaccines were implanted on it in separate areas (vaccine obtained from the Kaluga Biofabrika (Kaluga Biological Products Plant), prepared between 4 January and 19 March 1956). The implanted tests were placed in a thermostat for culturing, and for spore formation at a temperature of 34 to 35 degrees Centigrade for 6 days. Following this period of culturing the vaccines were tested for purity, typical growth and spore formation. Test tubes found to contain foreign bacilli or a characteristic growth were discarded, and samples consisting of pure vaccine with full quality spores were washed with physiological solution.

After washing, the vaccines were rinsed and centrifuged four times to free any radioactive sulfur adsorbed on the microbe bodies. A microbe suspension was prepared from the rinsed vaccine, containing 5 billion microbe bodies per milliliter at standard turbidity, and the radioactivity of this suspension per 0.1 ml was determined with the aid of an end counter.

In setting up the experiments for study of marked bacilli in the animal organism we bore in mind the period of time the vaccine remained in the organism, and the site of localization.

The experiments were performed on rabbits weighing 1,500 to 1,800 grams. Two series of experiments were performed.

In the first series 6 rabbits were injected subcutaneously with marked first vaccine of Tsenkovskiy. The inoculation consisted of 1 ml of vaccine, with 1.7 microcuries radioactivity. After a period of 8 days 1.5 ml of the second vaccine, with total radioactivity of 1.95 microcuries was injected. The control experiment consisted of 3 rabbits which were not vaccinated, but which were injected subcutaneously with radioactive sulfur with total activity of 2.15 microcuries. Samples of blood were taken from the peripheral vein of the ear on the 1st, 2nd, 3rd and 5th days in the case of the experimental rabbits, with subsequent samples taken on each successive 5th day, and the radioactivity of the blood samples was determined.

The average indicators of radioactivity of the blood of all the experimental rabbits were plotted on a curve.

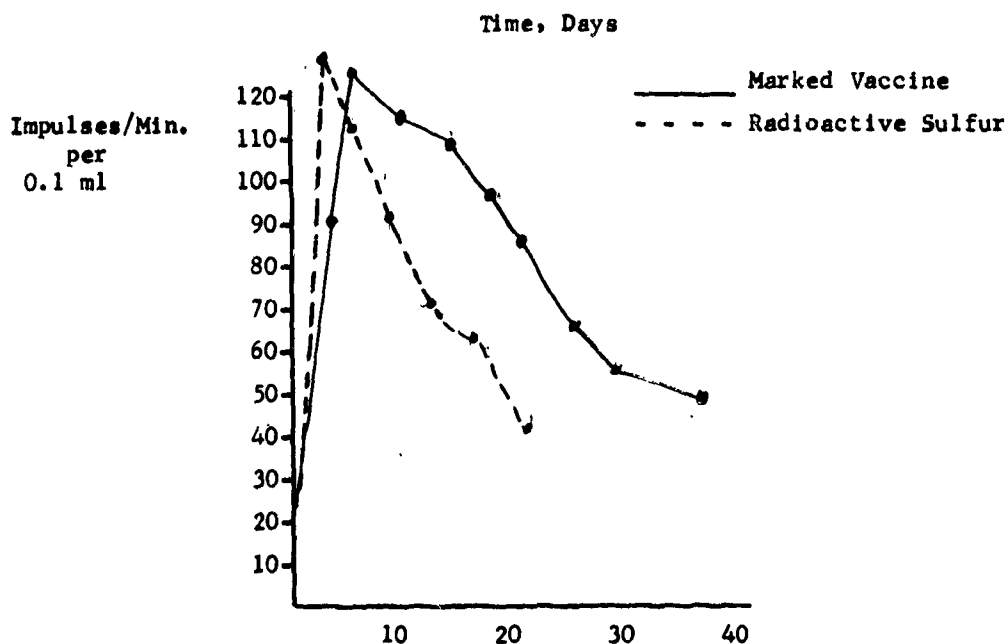


Figure 1. Change in concentration of radioactive sulfur in the blood following injection of marked Tsenkovskiy vaccine, and in one case of injection of radioactive sulfur.

As may be seen from Figure 1, following injection of the marked vaccines the radioactivity of the blood increased for two days, after which it decreases. The decrease in concentration of radioactive sulfur in the blood is gradual, dropping to the minimum, approximating the background level [see Note] during 35 days. The curve for the control rabbits, which had been injected with radioactive sulfur, only, differs from that of the rabbits receiving the marked vaccine. In this case, following a sharp increase in the radioactivity of the blood the concentration of radioactive sulfur drops rapidly, reaching the minimum value on the 20th day.

Thus the experimental results indicate that with subcutaneous injection of marked Tsenkovskiy vaccine in rabbits the radioactivity of the blood is displayed to the greatest extent during the first two days; on successive days it drops gradually, attaining the minimum on the 35th day, whereas the radioactivity of the blood of the control rabbits drops more rapidly, and the minimal amount of indicator is observed 20 days after injection of the radioactive sulfur.

([Note] Background: amount of impulses detected by the counter due to cosmic rays.)

The distribution of the marked vaccines also was studied according to organs. This experiment consisted of two parts: in the first part the distribution of marked vaccines in the organs of rabbits 20 days after vaccination was investigated, and in the second part, the distribution of vaccines containing radioactive sulfur was investigated according to individual organs of animals at the end of the experiment (35 days).

Two of the six immunized rabbits, and three control rabbits, in which the radioactivity level of the blood had dropped to minimum during this period, were killed 20 days after injection of the marked vaccines, for the purpose of determining the radioactivity of the organs. The concentration of radioactive sulfur per 100 milligrams organ weight was calculated.

The results obtained are shown in Figures 2 and 3.

Upon comparison of the radioactivity of the organs of rabbits immunized with marked vaccine, with that of the organs of control rabbits receiving radioactive sulfur revealed that the former had a higher level of radioactivity. The activity of 100 mg of liver or spleen of rabbits receiving marked vaccine was 210 to 230 impulses/minute, while the radioactivity of the same organs of the control rabbits was 48 to 55 impulses/minute, or four-fold less.

Furthermore, as may be seen from Figure 2 the distribution of marked antigen is irregular with respect to organs. In the rabbits receiving marked vaccine the greatest concentration of radioactive vaccine was found in the liver, less in the spleen and kidneys, and the least in the brain, muscles and bone marrow. This indicates that different sections of the reticulo-endothelial system vary with respect to their ability to absorb microbes, protein or other substances, and that in our experiment the greatest absorption was found to be in liver and spleen cells. With respect to the distribution of radioactive sulfur according to individual organs of control rabbits, a low level of radioactivity was found in all the tested organs on the 20th day after injection of the radioactive substance. The distribution of radioactive sulfur in the liver, spleen and kidneys was practically identical in all three rabbits. The concentration of indicator was lower in the muscles and in the brain, although it was higher in the bone marrow than in the other organs. This apparently is explained by the reticulo-endothelial system of the bone marrow.

At the end of the experiment, on the 35th day after injection of the marked vaccine, the 4 remaining rabbits were killed and their organs tested for resolution of the second part of the experiment, i.e., the period of storage and the distribution of marked vaccines according to individual organs of the animals.

The data of this experiment are presented in Figure 4.

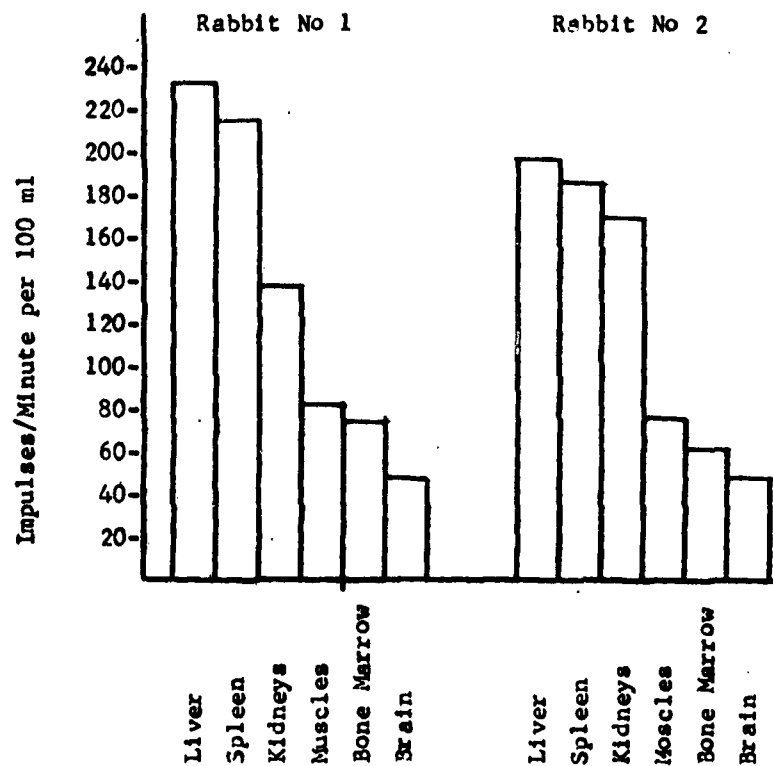


Figure 2. Distribution of marked vaccines according to organs of the rabbit, 20 days after immunization.

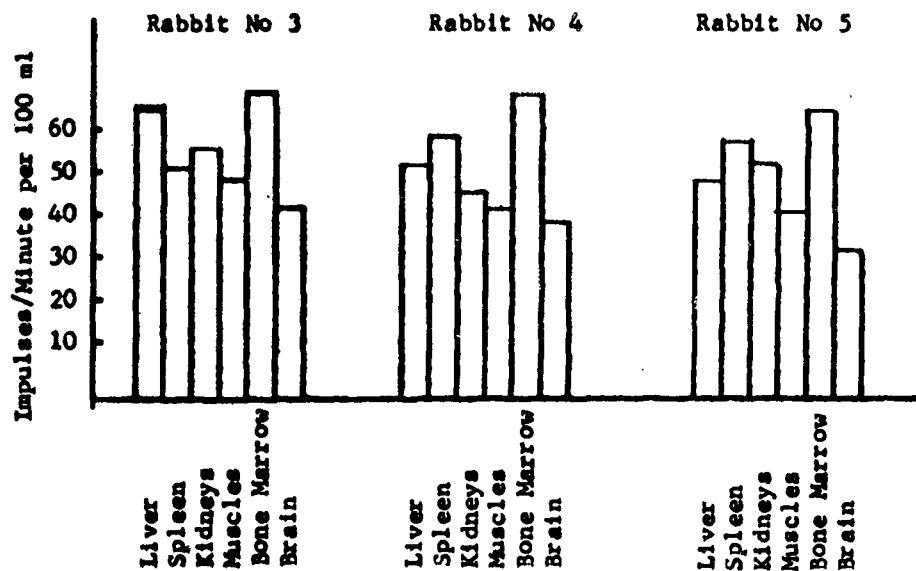


Figure 3. Distribution of radioactive sera according to organs of the rabbits, 20 days after introduction of indicator



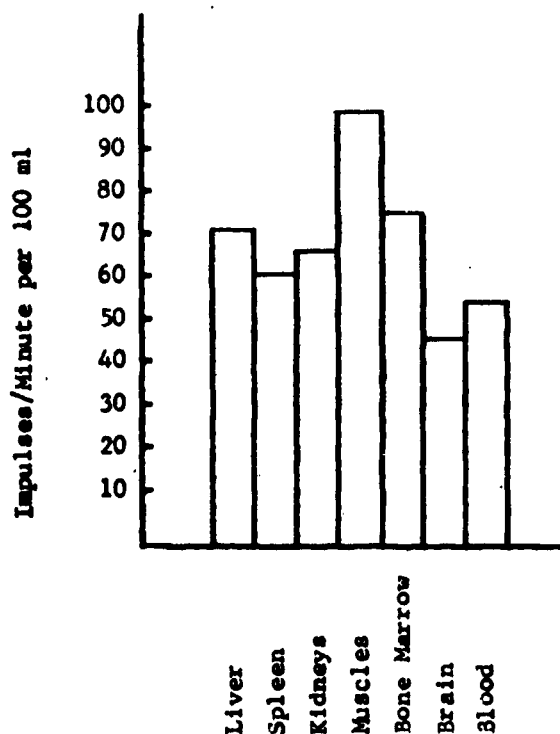


Figure 4. Distribution of marked vaccine according to organs of the rabbit at the end of the experiment.

Measurements revealed a low level of radioactivity in all the tested organs, and indicated that the concentration of radioactivity was approximately identical in the liver, spleen, kidneys and brain, with the highest level of radioactivity in the muscles.

Thus the average total radioactivity of the muscles was 88 impulses/minute, compared to 66 imp/min in the liver, spleen, kidneys and brain, i.e., the level of accumulation of marked vaccine in the muscles remained the same as in the rabbits killed 20 days after immunization. The identical content of marked vaccine in rabbit muscle after periods of 20 and 35 days apparently may be explained by the fact that the metabolism of muscle tissue of animals is much slower than in the cells of the reticulo-endothelial system.

Thus the experiment indicated, first, that on the 20th day after immunization, marked vaccines injected into the rabbit organism are found in greatest amounts in the liver, spleen and kidneys, with less in the brain, bone marrow and muscle; second, the accumulation of radioactive sulfur in control rabbits, which were not vaccinated, was considerably lower, or four-fold less, under the same conditions and during the same period of time; third, the least amount of marked vaccines was found in the organs of immunized rabbits 35 days after injection of the first and second Tsenkovskiy vaccines.

In application of the marked atom method the process of migration of microbe bodies, toxins or immunogenic substances in the blood or in the organs is of particular interest. What is the reason for the discovery of radioactivity in the blood and in the organs? The reason is the migration of microbe bodies or products of their decomposition, containing radioactive sulfur, or simply radioactive substances. Survival of *B. anthracis* of the first and second Tsenkovskiy vaccines in the liver, spleen or kidneys in the form of corpuscular bodies during the course of 20 days or more may hardly be postulated because according to data in the technical literature lysis and decomposition of bacilli occurs 6 to 8 days after injection of the vaccine.

In employing the method of marking bacterial with radioactive isotopes in the course of investigation of immunity to dysentery V. L. Troitskiy and his associates noted that in immunization of laboratory animals the radioactivity in the organs is caused by migration of whole antigens or immunogens, containing radioactive substance.

It is known that the whole antigen is a polysaccharide-lipoid-protein complex, which is obtained by extraction of bacteria with 0.25 N solution of trichloroacetic acid at low temperature. However, L. A. Zil'ber indicates that in obtaining whole antigens from bacterial masses not all the whole antigen contained in the mass is extracted, and that according to the investigations of Konnikov, Fradkina, Kholchev et al., the substrate remaining after extraction of the whole antigens has immunizing action. Therefore, following injection of first and second Tsenkovskiy vaccines into the rabbit organism the microbes undergo splitting and lysis, although this product of splitting of the microbe body may not be considered to be free from whole antigen or immunogen.

This means that the discovery of radioactivity in the blood and in the organs is due to the presence of immunizing substance, whole antigen containing radioactive sulfur, in the organs.

As indicated by our experiments, radioactivity of the blood and organs was retained 15 days longer in vaccinated rabbits than in control rabbits, thus this also indicates the presence of marked antigen (immunogen) in the blood and organs of the immunized animals.

In the second series of experiments it was important to study the dynamics of the distribution of marked anthrax bacilli in the immunized organism.

For the purpose of resolution of this task 6 rabbits were injected subcutaneously with the first vaccine of Tsenkovskiy, with 0.5 ml administered on the first occasion, followed by the same dosage of the second vaccine on the eighth day. These animals were inoculated with marked anthrax culture of virulent strain (strain No 575) 30 days after vaccination. The strain B. Anthracis was obtained from the Republic Veterinary Bacterial Laboratory (Respublikanskaya vetbaklaboratoriya); prior to inoculation this culture was calibrated in rabbits, and minimum fatal dosage was established. The culture, in the form of a microbial suspension, was injected subcutaneously in a concentration of  $10^9$  microbes per milliliter, and a radioactive concentration of 1.6 microcuries.

By way of control, 3 non-immunized rabbits were injected with radioactive sulfur at the same level of activity (1.6 microcuries).

The data on the average indicators of radioactivity of the blood of all the experimental animals are presented in Figure 5.

After inoculation with the marked culture and injection of the radioactive sulfur samples of blood were taken daily from peripheral veins of the ear and the radioactivity was determined. The investigations revealed that the radioactivity of the blood of the immunized rabbits, inoculated 30 days after vaccination, dropped relatively rapidly, and on the 8th day decreased to the minimum, the background level.

The decrease in concentration of indicator in the blood of the control rabbits took a much longer period, 16 days.



Figure 5. Change in concentration of radioactive sulfur in the blood of rabbits following inoculation with marked anthrax culture.

After these results were obtained in the investigation of the blood the experimental rabbits were killed and their organs were subjected to tests for presence of the indicator.

The results of the experiment are shown in Figures 6 and 7.

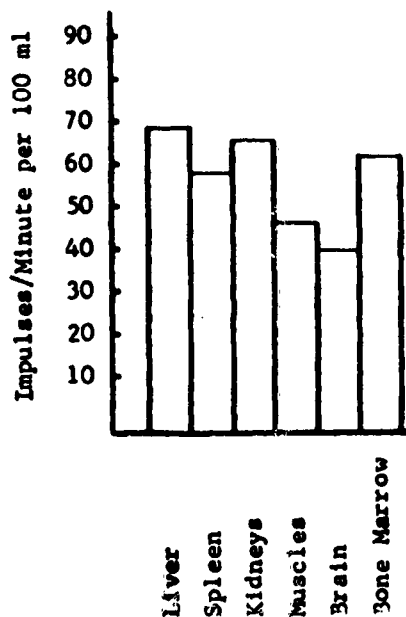


Figure 6. Distribution of marked anthrax bacilli in the organs of immunized rabbits.

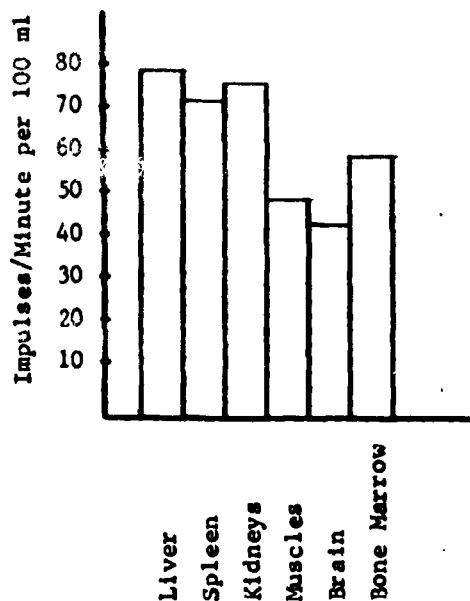


Figure 7. Distribution of radioactive sulfur in the organs of rabbits.

Measurement of the radioactivity of the organs of immunized rabbits killed on the 8th day after inoculation revealed considerably lower radioactivity and irregular distribution of marked anthrax bacilli according to organs.

In investigating the organs of the non-immunized rabbits, killed on the 16th day after injection of radioactive sulfur, it was found that the concentration of radioactive substance was somewhat higher, and the character of its distribution was the same as in the organs of the immunized rabbits.

Thus the investigation of the killed animals revealed that the data obtained coincide with the radioactivity indicators of the blood of the immunized and control rabbits.

On the basis of the foregoing experiments it may be concluded that the immunized organism liberates itself of injected virulent anthrax bacillus much more rapidly than does the non-immunized organism.

Thus the dynamics of the change of blood radioactivity and radioactivity of the organs of animals immunized against anthrax indicate that the causative agent of anthrax does not remain for a long time in the immune organism, which indicates strengthening of phagocytic activity of the reticulo-endothelial system in immunized animals.

The basic phagocytic theory presented by I. I. Mechnikov in his lectures in 1891 at the Pasteur Institute indicate that he and his associates observed leucocytosis, and absorption of the causative agent of anthrax by leucocytes in the case of anthrax.

S. N. Vyshel'skiy, who attributes great importance to leucocytosis in the anthrax process, considers that an increase in the leucocytes inhibits the development of septicemia.

In investigating phagocytic activity of leucocytes in animals immunized against anthrax, A. B. Boyakhchyan established that leucocytosis increases in the immune organism.

The results of our experiments also support these data on the active protective function of the immune organism against the anthrax bacillus.

On the basis of these investigations we consider that the problem of whether the radioactivity observed in the blood and organs is caused by the presence in the blood and organs of marked vaccines or radioactive sulfur which may be liberated from the microbe cells as a result of their death and lysis, has not been finally resolved.

Extraction of the polysaccharide-lipoid-protein complex, which is considerably stable with respect to enzymatic splitting, from the anthrax bacillus is a task for future investigation. This marked complex remains undestroyed in the organism for a fairly long period of time, enabling study of several problems of the pathogenesis of anthrax.

#### CONCLUSIONS

1. Marked bacilli of anthrax first-, and second vaccines were obtained by growing the first-, and second Tsenkovskiy vaccines on glycerine-peptone agar containing radioactive sulfur in the form of methionine. We used the marked vaccines for study of their distribution in the animal organism.

2. Upon subcutaneous injection of marked Tsenkovskiy vaccine in rabbits the radioactivity of the blood increased the most during the first two days, and on subsequent days gradually decreases and arrived at the background level on the 35th day, while in the case of the control rabbits, which were injected with radioactive sulfur, only, the activity of the blood dropped sooner, and the minimal level of the indicator in the blood was attained 20 days after injection of the radioactive substance.

In determining the activity of the organs of the animals, a high level of radioactivity of the liver, spleen and kidneys was observed 20 days after injection of the marked vaccines, although the radioactivity of these organs was four-fold lower in the control rabbits.

4. At the end of the experiment, i.e., on the 35th day after injection of the marked vaccine, a low level of radioactivity was noted in all the organs tested. The distribution of radioactivity according to organs was approximately identical.

5. In the immunized animals, with subcutaneous injection of marked, virulent anthrax bacilli the radioactivity of the blood dropped rapidly, and on the 8th day attained the minimum, while in the case of the control rabbits the indicator remained in the blood for a much longer time, i.e., approximately twice as long.

6. On the 8th day following injection of marked, virulent anthrax culture a considerable reduction of radioactivity of the organs was noted in the immunized rabbits, and the distribution of radioactivity according to organs was irregular. In the investigation of the organs of the control rabbits killed on the 16th day after injection of the indicator the concentration of radioactive sulfur was found to be somewhat higher than in the immunized animals. On the basis of the data obtained it may be concluded that the immunized organism liberates itself of injected radioactive anthrax bacilli considerably faster than the nonimmunized organism rids itself of radioactive sulfur.

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